Interim Guidance on Assessing the Risk Posed by Pathogens Associated with Dredged Material

by Karl J. Indest

PURPOSE: This technical note provides guidance to U.S. Army Corps of Engineers Districts in assessing the risk posed by pathogens associated with dredged materials.

INTRODUCTION: Management of contaminated sediments has focused predominantly on chemicals, whereas potential risks posed by pathogenic biological contaminants are assessed on an ad hoc basis. Currently the Inland Testing Manual (U.S. Environmental Protection Agency (USEPA) 1998) addresses pathogenic concerns about sediments as they relate to Clean Water certification requirements. The three major areas of concern identified for microbiological contamination and effects related to dredged sediments are (1) contamination of harvestable shellfish, (2) body contact through recreational use, and (3) contamination of drinking water. While programs and guidelines are in place to regulate discharges of biological wastes, programs or guidelines to evaluate the impacts of pathogens in sediments are rudimentary. That is likely to change now that the Clean Water Act has been amended to include specific language regarding assessment of pathogens and pathogen indicators. The amendment, known as the Beaches Assessment and Coastal Health (BEACH) Act of 2000, requires that states adopt USEPA-approved coastal recreational water quality criteria and standards for pathogens and pathogen indicators by April 2004. The BEACH Act also designates USEPA to study issues associated with pathogens and public health and to publish new or revised criteria by October 2005. In response to the BEACH Act, USEPA has outlined a waterborne microbial disease strategy with the overall stated goal of "protecting human health against exposures to harmful levels of pathogens in ground and surface water, food sources, and drinking water" (USEPA 2001a). As part of this strategy USEPA has identified sediments as a potential source of pathogens. Parallel to these efforts USEPA also has published a protocol for developing pathogen Theoretical Maximum Daily Loads (TMDLs) (USEPA 2001b).

A main driver behind the amendment to the Clean Water Act as well as USEPA's waterborne microbial disease strategy initiative is the steady increase in incidences of pathogen indicators in recreational waters. The Natural Resources Defense Council's annual "Testing the Waters" report showed an 83 percent increase in the number of beach closings due to elevated pathogen indicators in 2001 (Natural Resources Defense Council 2001b). Elevated pathogen indicators in recreational waters translate into significant health and economic costs. The Centers for Disease Control and Prevention (CDC) estimates that close to a million cases of illnesses occur annually in the United States as a result of exposure to waterborne microbial pollution. Most of these illnesses will be self-limiting but as many as 1 percent may result in death. In addition to the human health cost, significant economic losses can be attributed to microbial pollution. USEPA (2000b) estimates that coastal waters support 28.3 million jobs and generate $54 billion in goods and services each year. Elevated pathogen indicators in recreational waters threaten these revenues.

PATHOGENS AND PATHOGEN INDICATORS: Pathogens commonly associated with waterborne diseases can be grouped into three main categories: bacteria, protozoans, and viruses (Table 1). Bacteria are prokaryotic unicellular organisms that lack a nucleus and reproduce by binary fission (Brock and Madigan 1991). In contrast, protozoans are unicellular eukaryotes that lack cell walls (Brock and Madigan 1991). Viruses, conversely, are infectious particles that require a living host to divide. Pathogenic protozoans and viruses are of particular concern because they can persist in the environment for extended periods of time.

Table 1
Pathogens Associated with Waterborne Diseases

Pathogen Name	Pathogen Type	Disease
Escherichia coli 0157:H7	Bacteria	Gastroenteritis
Salmonella typhi	Bacteria	Typhoid fever
Salmonella	Bacteria	Salmonellosis
Shigella	Bacteria	Shigellosis
Vibrio cholera	Bacteria	Cholera
Yersinia enterolitica	Bacteria	Yersinosis
Cryptosporidium	Protozoan	Cryptosporidiosis
Giardia lamblia	Protozoan	Giardiasis
Enterovirus	Virus	Gastroenteritis
Hepatitis A	Virus	Infectious hepatitis
Adenovirus	Virus	Gastroenteritis
Calicivirus	Virus	Gastroenteritis

Most of the pathogens in Table 1 are associated with animals as well as humans. Cattle, for example, can serve as a major reservoir for *E. coli 0157:H7*, *Cryptosporidium*, and *Giardia* (Cole et al. 1999). Chickens can harbor the pathogenic bacteria *Salmonella* and *Campylobacter* (Cole et al. 1999). The majority of these agents are linked with gastrointestinal illnesses. The severity of disease can greatly fluctuate depending on the virulence of the agent and the age and immune status of the human host. In general children and the elderly are at a greater risk of developing life-threatening complications associated with exposure to pathogens from recreational and drinking waters.

For more than a century, pathogen indicators such as *Escherichia coli* have been used to evaluate the microbial safety of drinking water. Because types of waterborne pathogens are highly variable and they are present in minute concentrations in the environment, indicator organisms are used as surrogates for developing indices of bacteriological water quality. Pathogen indicators are nonpathogenic bacteria that are usually associated with pathogens transmitted by fecal pollution. These indicators are present in high numbers as part of the normal mammalian gut flora and are easily sampled and measured. Common indicators that have been used to develop water quality criteria to support various designated uses are outlined in Table 2 (modified from USEPA 1986). USEPA publishes these criteria under 304(a) guidelines to states and tribes.

Table 2
Indicators Used to Develop Water Quality Criteria

Designated Use	Pathogens Evaluated	Criteria
Recreation	*E. coli*	Fresh water: Geometric mean of 126 CFU per 100 mL, based on not less than 5 samples equally spaced over a 30-day period.
	Enterococci	Fresh water: Geometric mean of 33 CFU per 100 mL, based on not less than 5 samples equally spaced over a 30-day period. Marine: Geometric mean of 35 CFU per 100 mL, based on not less than 5 samples equally spaced over a 30-day period.
	Fecal coliform	Geometric mean of 200 CFU per 100 mL, based on not less than 5 samples equally spaced over a 30-day period and no more than 10 percent of the samples exceeding 400 CFU per 100 mL during any 30-day period.
Shellfish harvesting waters	**Total coliform**	Geometric mean of 70 MPN per 100 mL, with not more than 10 percent of the samples taken during any 30-day period exceeding 230 MPN per 100 mL.
	Fecal coliform	Median concentration should not exceed 14 MPN per 100 mL with not more than 10 percent of the samples taken during any 30-day period exceeding 43 MPN per 100 mL.
Public drinking water	**Total coliform**	Ninety percent of all daily raw water samples taken contain no greater than 100 CFU/100 mL for surface water systems to remain unfiltered.
	Fecal coliform	Ninety percent of all daily raw water samples taken contain no greater than 20 CFU/100 mL for surface water systems to remain unfiltered.
	E. coli	Ninety percent of all daily raw water samples taken contain no greater than 20 *E. coli* CFU/100 mL for surface water systems to remain unfiltered.

Note: CFU = Colony forming unit.
MPN = Most probable number.

PATHOGEN SOURCES: By and large the most significant source of waterborne pathogens is the expanding human population. This situation is being exacerbated in coastal regions where it is estimated that half of the human population of the United States will reside by 2010 (Natural Resources Defense Council 2001b). Sewage overflows from outdated and aging combined and sanitary sewers along with malfunctioning sewage treatment plants and pump stations continue to serve as main point sources of pathogens in coastal waters. Onsite septic systems are also a significant source of waterborne pathogens. Fecal contamination originating from these systems can travel considerable distances through soil, eventually contaminating ground and surface waters. It has been estimated that nationwide there are some 25 million septic tanks receiving 175 billion gallons of human wastewater (American Society of Microbiology 1999b), 10-30 percent of which are not functioning properly (USEPA 2001a).

In addition to human sewage inputs, animal waste is also a significant source of waterborne pathogens. The American farm has changed dramatically over the last 60 years. Scientific advancements and new technologies have given way to Concentrated Animal Feeding Operations (CAFO). Collectively, these facilities generate 220 billon gallons of waste annually (Natural Resources Defense Council 2001a). Many of these facilities are located in floodplains, increasing the likelihood of introducing pathogens into watersheds. In addition to the sheer quantity of pathogens produced by CAFOs, there is some concern about the quality of pathogens generated by CAFOs. Animal waste routinely contains pathogenic organisms such as *Cryptosporidium* and *Campylobacter,* organisms not normally associated with human waste (Cole et al. 1999). Furthermore, the widespread use of subtherapeutic concentrations of antibiotics in animal feed has led to increased incidences of antibiotic resistance among microbial isolates derived from animal

waste (American Society of Microbiology 1999a). The proliferation of antibiotic-resistant microorganisms has been recognized by the CDC as a serious public health concern.

Storm water runoff further magnifies the levels of pathogens and pathogen indicators introduced into watersheds through nonpoint sources. Pollution by urban storm water accounts for about a quarter of the Nation's contaminated estuaries and lakes as well as a significant source of beach pollution (Natural Resources Defense Council 2001b). Agricultural runoff contains, in addition to pathogens, high concentrations of nutrients and pesticides. Nutrient pollution can lead to eutrophication of waterways and stimulate the growth of organisms like *Pfiesteria,* which was responsible for the death of more than a billon fish in the Chesapeake Bay region (American Society of Microbiology 1999b).

SURVIVAL OF PATHOGENS AND THEIR INDICATORS: Numerous studies have been conducted on the survival rates of different pathogens and their indicators in various environmental matrices (Burton 1985). Microbial survival is commonly expressed using exponential decay equations of the sort $dN/dt = -KN$, where t = time, K = rate constant, and N = number of microorganisms (Hurst 1997). In practice, however, long-term survival of pathogens and pathogen indicators is more significant than predicted by first-order decay equations. The reason for this is that various environmental factors such as sunlight, temperature, pH, salinity, and predation affect survival (Roszak and Colwell 1987). In general, it appears that cooler freshwater environments devoid of sunlight prolong survival of pathogens and their indicators. In addition, sediments have been shown to greatly extend the survival of pathogens (Davies et al. 1995; Sherer et al. 1992; LaLiberte and Grimes 1982; Burton, Gunnison, and Lanza 1987). Once pathogens enter the water column, they may become associated with various suspended solids that eventually settle out and accumulate in the underlying sediments. Both bacteria and viruses possess electrostatic charges, which facilitates their adsorption onto fine-grained high-organic charged clays and muds. Sediments can contain 100 to 1000 times as many pathogen indicators as the overlying water (Grimes 1975, 1980). Sediments extend survival of pathogens and pathogen indicators because they provide nutrients as well as protection from predation. Due to the accumulation of pathogens in bottom sediments, resuspension of sediments can result in desorption of pathogens from sediments and the subsequent contamination of the overlying surface waters. Activities that resuspend sediments such as storm water events, wave action, tides, recreational use, and dredging can lead to transient increases in pathogens and pathogen indicators in the water column (Grimes 1975, 1980; Kebabijian 1994).

CURRENT METHODS: Standard methods traditionally used for measuring the level of pathogen indicators in water are generally not appropriate for sediments. These methods must first be modified to include steps to elute into suspension the bacterial fraction attached to the sediment. Sediment elutriates are normally prepared by combining 4 parts water to 1 part sediment by volume followed by some form of dispersion for a defined period of time (USEPA 1998). Generally three main methods of dispersion have been used: chemical, sonication, or homogenization (Van Elsas and Smalla 1997). Of the three methods, sonication has been shown to be by far the most effective means for the separation of bacteria from sediment particles (McDaniel and Capone 1985). Following dispersion, the water phase is separated from the sediment by settling and/or gentle centrifugation. Once the elutriate has been prepared, it should be used immediately. Estimates of pathogenic contamination can be determined from sediment elutriates using standard pathogen

indicator methods as described in Table 2. Of the indicators listed, USEPA recommends using *E. coli* and *Enterococci* as indicators of fecal contamination in fresh waters and marine waters, respectively.

Two general methods are used to enumerate fecal indicator organisms: the most-probable-number (MPN) technique and the membrane filter technique (MF) (Rompre et al. 2002). In both cases, results are reported in colony forming units (CFUs), which correspond to the number of viable pathogen indicator cells per unit volume. The MPN method is an older technique that consists of inoculating a series of tubes containing lactose or lauryl tryptose broth with various dilutions of the water sample. Products of lactose fermentation such as gas production or acid formation in the test tubes after 48 hr incubation at 35 °C is considered a presumptive positive. The accuracy and speed of this method have been improved by adding chromogenic and fluorogenic substrates to the media to assay for specific enzymes unique to certain bacterial indicators. The number of bacteria present in a sample is expressed as MPN, which is a statistical estimate of the mean number of organisms in the sample.

The MF technique is faster and more accurate than the MPN method and is therefore recommended by USEPA for water monitoring. MF consists of filtering a water sample through a sterile filter with a 0.45-μm pore size, which retains bacteria. This filter is subsequently incubated on a selective medium and the number of bacteria are enumerated. Detailed protocols using this method are available at the USEPA Web site (USEPA 2000a). As with the MPN technique, to increase the accuracy and speed of the MF method, chromogenic and fluorogenic substrates have been added to the media to assay for specific enzymes unique to certain bacterial indicators. Currently a number of commercial kits on the market are based on both of these methods (Appendix I). These methods have been optimized to get results within 24 hr.

CURRENT LIMITATIONS: Recently, there has been a resurgence in criticisms questioning the suitability of bacterial indicators as markers of microbial water safety (Leclerc et al. 2001; Rose and Grimes 2002). Advances in the bacteriology of the coliform group have revealed that thermotolerant fecal coliforms can have an environmental origin, making them unsuitable as an indicator of fecal contamination (Leclerc et al. 2001). In addition, these organisms exhibit a high regrowth potential once introduced into the environment (Leclerc et al. 2001). For these reasons USEPA now recommends using *E. coli* and *Enterococci* as indicators of fecal contamination in fresh waters and marine waters, respectively. Recovery of these indicators from the environment is still problematic because they can enter a viable but nonculturable state (Rollins and Colwell 1986). Under these conditions, the indicator method leads to a high incidence of false negatives underestimating the actual number of indicator organisms present.

Despite improvements in the standard methods, the indicator concept is still being challenged on the grounds that the environmental fate of bacterial indicators differs from that of the pathogens they proxy for (Rose and Grimes 2002). The current methodology used to predict the presence of pathogenic microorganisms in water is based on the observations that as the number of fecal indicator organisms increase in, for example, a recreational water, the number of gastrointestinal illnesses increase. That is to say there is a correlation between the number of fecal indicator bacteria present in a water medium that people are exposed to and the incidence of gastrointestinal disease. Recent observations, however, indicate that the incidences of certain classes of pathogens, such as

protozoans and viruses, do not correlate with the incidences of pathogen indicators. A study looking at coastal waters off southern California showed that the incidence of various bacterial indicators (fecal coliforms and enterococci) did not correlate with the presence of human adenoviruses (Jiang, Nobel, and Chu 2001). Viruses and protozoans appear to be more resistant to environmental stress and hence can exist in the environment for longer periods of time than indicator bacteria. Despite the shortcomings with these methods, they continue to be used today because they are well established, cost-effective, and easy to perform, and perhaps most importantly, no universally accepted alternative methods currently exist.

FUTURE RESEARCH: The future of water quality monitoring will likely rely on a matrix approach placing less emphasis on any one single parameter. The toolbox of potential new methods for detecting waterborne pathogens is extensive yet largely untested for environmental application. Nucleic acid technologies currently used in clinical medical research offer powerful alternatives to traditional indicator methods. Methods based on real-time polymerase chain reaction (RT-PCR) and DNA microarray technology offer a high-throughput format for analyzing multiple pathogen species in a single assay format (Lucchini, Thompson, and Hinton 2001; Pommepuy and Le Guyader 1998; and Toze 1999). These technologies provide speed and accuracy while eliminating the need for culturing organisms that in some cases can be very tedious if not impossible. Research is currently engaged in adapting/modifying these technologies to be used in assessing the risks posed by pathogens in sediments. Along these lines the sensitivity of a commercially available RT-PCR based TaqMan® *E. coli* 0157:H7 amplification/detection kit was evaluated. The sensitivity of this kit was empirically determined using 0.1-gram sediment samples that were spiked with known amounts of *E. coli* 0157:H7 DNA. The results seen in Figure 1 show a divergence of the data set from linearity occurring at about 1000 CFUs. Thus, the lower detection limit for this assay under the conditions employed is 1000 *E. coli* 0157:H7 cells in 0.1 gram of sediment.

For comparison, a parallel experiment was conducted looking at the sensitivity of the assay under conditions in which pure water was spiked with known amounts of *E. coli* 0157:H7 DNA. The results seen in Figure 2 show a divergence of the data set from linearity occurring at a lower value of 100 cells or CFUs, suggesting a significant inhibitory matrix effect by the sediment on the sensitivity of the assay. This 10-fold inhibitory effect is likely to be exacerbated upon processing of increased sediment sizes. Currently additional experiments are being conducted to determine the upper limit of sediment sample size that can be processed with the maximum sensitivity. A quantitative understanding of matrix effects on assay performance is necessary because pathogens, unlike their indicators, are present in only small quantities in the environments. To compensate for this low cell abundance, more sediment must be extracted, which in turn can negatively impact assay sensitivity. In addition, caution should be used when interpreting data generated by these methods. Because these technologies are mostly based on DNA detection of an agent, DNA from dead organisms may be detected by these methods. For this reason these methods normally indicate "pathogen potential."

Information gained from these new approaches can provide valuable input to environmental risk assessment determinations. Currently, the limitation has been how the biological relevance of DNA data is interpreted in a risk framework. Recently, a study was published that looked at developing a framework for interpreting PCR results obtained from water matrices in the context of risk to human health (Loge, Thompson, and Call 2002). The study concluded that while the method was valid in
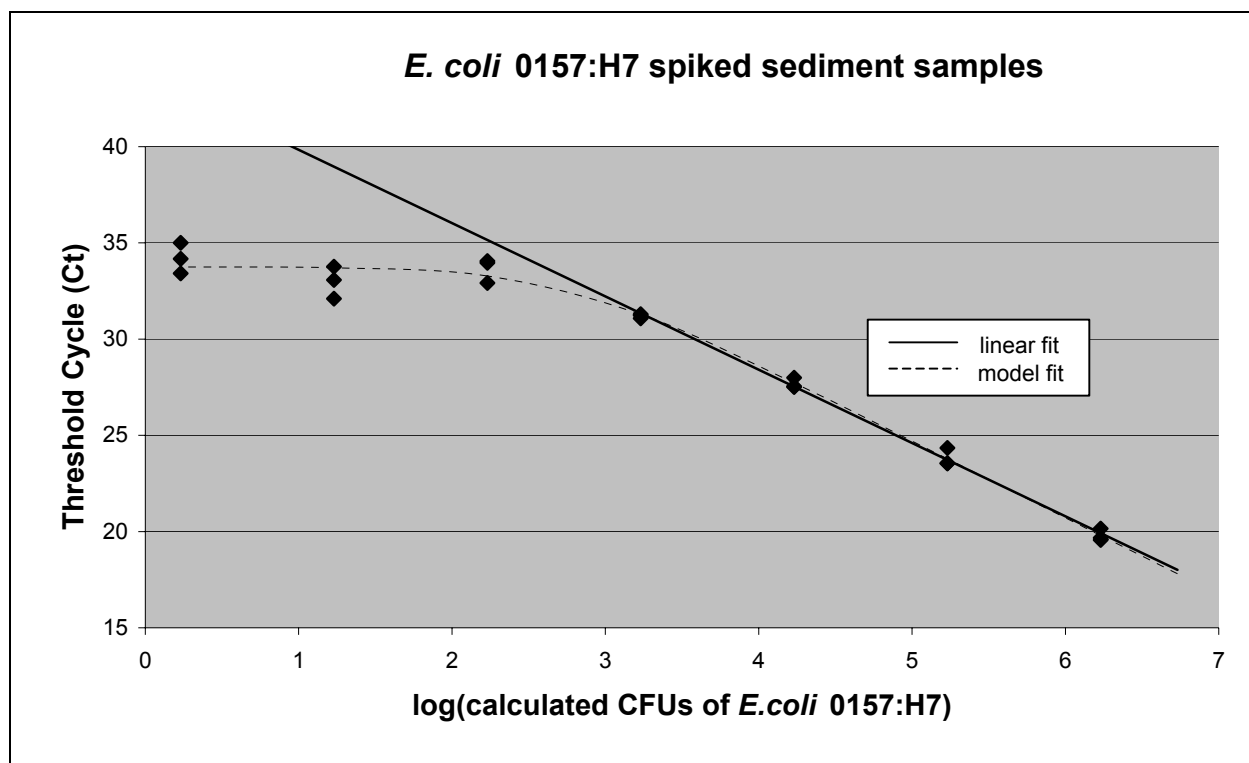
E. coli 0157:H7 spiked sediment samples

Figure 1. Detection limit of TaqMan® assay using *E. coli* 0157:H7 spiked sediment samples. 100-mg samples of sediment were spiked with 10-fold serial dilutions of *E. coli* 0157:H7 DNA ranging from 10^{-8} to 10^{-14} grams. DNA from spiked samples was extracted using MO BIO DNA soil extraction kits. Resulting extracts were analyzed using ABI's *E. coli* 0157:H7 TaqMan® detection kit according to the manufacturer's instructions. A lower detection limit of 1000 cells or CFUs was calculated based on the graphical divergence from linearity. Theoretical CFUs were calculated based on the mass of a genome from a single *E. coli* 0157:H7 cell. See text for details

some cases, significant improvements were needed in sample processing and filtration. In addition to technological improvements, exposure pathways and dose-response data for most waterborne pathogens in sediment matrices are either lacking or incomplete. As a result, current risk assessments for pathogens rely heavily on computer modeling and poorly defined epidemiological data. Despite these limitations researchers are actively engaged in applying risk-based methods toward the interpretation of nucleic acid data.

CONCLUSIONS: In an attempt to provide interim guidance to U.S. Army Corps of Engineers Districts in assessing the risk posed by pathogens in dredged material, the scope of the problem and current scientifically defensible methods were reviewed. It is apparent that the recent regulatory and scientific climates surrounding the issue of pathogens are very dynamic and very much in transition. While the current methods are imperfect, there are to date no universally accepted alternatives with which to replace existing ones. New technologies based on PCR and DNA array technology promise to be powerful alternatives for pathogen detection, yet interpretation of molecular data in a risk assessment context has been problematic. In the meantime, the current imperfect methods are being relied upon more and more to make increasingly complex regulatory decisions. Issues such as pathogen TMDLs are likely to come to the forefront as agencies struggle to address controls of microbial contamination in watersheds.
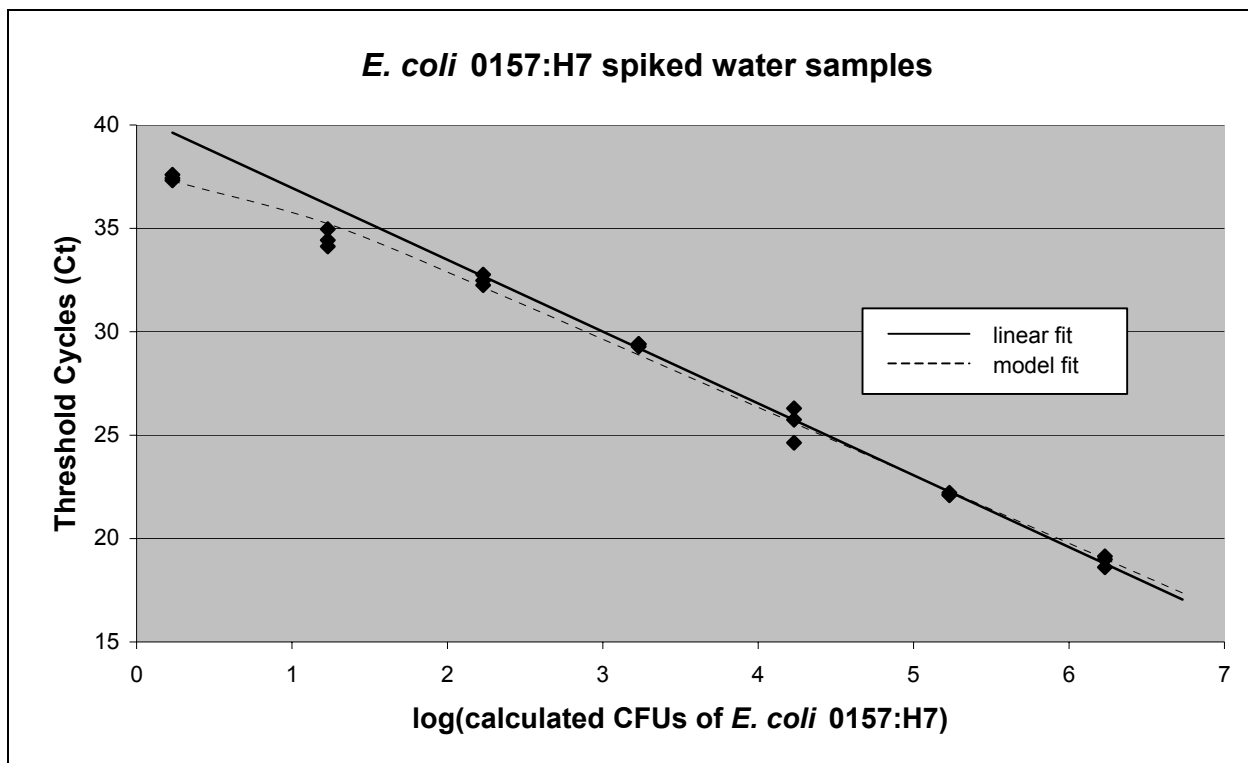
Figure 2. Detection limits of TaqMan® assay using *E. coli* 0157:H7 DNA spiked in pure water. Ten-fold serial dilutions of *E. coli* 0157:H7 DNA ranging from 10^{-8} to 10^{-14} grams of DNA were added to pure water. Samples were analyzed using ABI's *E. coli* 0157:H7 TaqMan® detection kit according to the manufacturer's instructions. A lower detection limit of 75-100 cells or CFUs was calculated based on the graphical divergence from linearity. Theoretical CFUs were calculated based on the mass of a genome from a single *E. coli* 0157:H7 cell. See text for details.

POINTS OF CONTACT: For additional information, contact the author, Dr. Karl J. Indest ((601) 634-2366, Karl.J.Indest@erdc.usace.army.mil), or the Manager of the Environmental Effects of Dredging Programs, Dr. Robert M. Engler ((601) 634-3624, Robert.M.Engler@erdc.usace.army.mil). This technical note should be cited as follows:

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APPENDIX I
COMMERCIALLY AVAILABLE REAGENTS

Commercially available reagents for the rapid identification and enumeration of *E. coli* as well as enterococci in water samples.

- Colilert® (INDEXX, http://www.idexx.com/Water/Products/index.cfm)

- Colilert-18® (INDEXX, http://www.idexx.com/Water/Products/index.cfm)

- Colisure® (INDEXX, http://www.idexx.com/Water/Products/index.cfm)

- m-ColiBlue24®
  (Hach, http://www.hach.com/Prod/microbiology.htm#Membrane)

- ColiComplete®
  (BioControl, http://www.rapidmethods.com/products/colicomplete.html)

- Microsure®
  (Gelman, http://www.pollardwater.com/emarket/Pages/L1604120media.asp)

- Enterolert® (INDEXX, http://www.idexx.com/Water/Products/index.cfm)

- Chromocult® enterococci agar
  (Merck, http://pb.merck.de/servlet/PB/menu/1020630/index.html)

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